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PEST MANAGEMENT GRANTS FINAL REPORT

Contract Number: 98-0262

Contract Title: Mass Releases of Natural Enemies of Vine Mealybug

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ABSTRACT:

This is an eleven-month report (effective April 1999) on an effort by Coachella Valley table grape growers, a commercial insectary, University of California, Riverside researchers and extension personnel to implement a long-term reduced-risk pest management system to control recently introduced vine mealybug (VMB) pests and promoting this approach to all growers and interested parties.

Progress includes: (1) Collaborators have provided all resources needed to establish and operate viable study/validation sites. (2) Insectary parasite production techniques have been modified resulting in up to a 25 times increase in numbers of parasites produced than in previous years, also with significant reduction in contamination of rearing colonies. The significant increase in production of parasites greatly enhances the potential to control VMB with biological control colonization and/or augmentation programs. (3) Significant increases in the VMB parasite *Leptomastidea* were found following parasite releases in spite of extremely hot temperatures. (4) We found a close relationship between VMB and ant activity, supporting the expectation that ant control will enhance parasite impact against VMB. (5) Using a modified ground rig, ants were controlled effectively for several weeks without affecting parasite numbers.

The progress reported above provide the basis for effective control of VMB in the spring preceding harvest.

EXECUTIVE SUMMARY

The vine mealybug (VMB) was first discovered in the Coachella Valley in Southern California in 1994. It has spread rapidly, causing severe economic damage to table grape vineyards in the region. More recently, it was discovered in the San Joaquin Valley. The University of California Cooperative Extension Viticulture Advisor for Kern County stated, "...at the present time, table grapes in the Coachella Valley are severely impacted, however the spread of this pest into the central valley now exposes over 600,000 acres of grapes to this problem"

Due to the lack of effective native parasites, a dramatic increase in the application of organophosphates (Chlorpyrifos, Diazinon, and Dimethoate) and Carbamates (Carbaryl and Methomyl) has been implemented to control the vine mealybug. Each of these pesticides is on the EPA's list of chemicals to be reviewed as required by the Food Quality Protection Act (FQPA) of 1996. Although the USDA and EPA have not listed table grapes as a commodity whose production depends heavily on the pesticides included on the priority list, table grape production will be adversely impacted if the above listed materials are not available. Regardless of the status of these chemicals, a biological, sustainable solution to the devastating VMB is necessary. In essence, there is no effective control for VMB currently available because even pesticides are only marginally effective due to VMB's living under the bark until fruit formation and the protection of VMB by ants.

The objectives for this proposal are to bring together a voluntary effort by Coachella Valley table grape growers, a commercial insectary, University of California, Riverside (UCR) researchers, and extension personnel for the purpose of implementing a long-term, reduced-risk pest management system for the control of vine mealybug. This will be done by mass-rearing parasites in a commercial insectary, releasing them in commercial vineyards, evaluating their establishment and effectiveness, and widely disseminating the results to encourage others to adopt this IPM approach.

The IPM grape group is well established and functioning effectively. All land and facilities for the program have been provided by growers in the Coachella Valley. The experimental design consists of approximately 8 acres in each of 4 farms with 5 treatments on each farm: (1) no treatment of any kind (reference data); (2) ant control only; (3) ant control + parasite releases; (4) parasite releases only; and (5) grower commercial applications.

Rearing and releasing of parasites is proceeding exceptionally well, much better than expected. We expect production to exceed 100,000 of each parasite species per week, a figure much higher than ever reported previously, anywhere. Production has exceeded expectations because of improvement in food (host plant) provided to VMB, and significant improvement in rearing techniques and equipment, including special equipment to greatly reduce contamination. Releases in 1999 over an 8 week period exceeded an average of 67,000/wk *Anagyrus* and 43,000/wk *Leptomastidea*. In 2000 from February-March over a 7 week period releases of *Anagyrus* have exceeded an average of 288,000/wk and 54,800/wk *Leptomastidea*. We easily expect parasite releases of over 100,000/wk of each species during April-May the most critical period to prevent damage by VMB in the Coachella Valley.

Ant control and monitoring of ant species and numbers is producing better than expected results. Ants are being treated with Lorsban using a specially designed spray rig that effectively kills ants without affecting parasites. With pitfall traps and visual observations we have recorded greatly reduced ant numbers in treated areas, up to 4 weeks effective reduction on vines.

From yellow sticky trap data on evaluation of parasites impact on VMB and impact of ants on parasites we have found the following: parasites survive, increase in numbers, and spread to non-release areas, up to 2 months after being released, including periods of time when temperatures are extremely high (over 110°F). Ants are present in high numbers and with very aggressive behavior and are closely associated with VMB. In results from visual samples over 8 weeks this spring (February-March) we find a strong trend indicating lowest numbers of VMB where we have treated for ant control or where parasites have been released. Notably higher numbers of VMB are being found in the commercial plots where there have been no parasite releases or ant control.

In summary, significant progress has been made in each of the 3 objectives in this proposal. In objective 1, a group of collaborators has provided all resources needed to establish viable study/demonstration sites and outside interested people and organizations are involved in the progress of this study. In objective 2, insectary parasite production techniques have been modified resulting in a great increase in numbers of parasites produced and released, also with significant reduction in the potential for contamination of insectary rearing colonies. In objective 3, we have demonstrated significant increases in *Leptomastidea* recovered following parasite releases under extremely hot temperatures. We have documented a close relationship between VMB and ant activity, supporting our expectation that effective ant control will enhance parasite impact against VMB. We have tested several baits for ant control and are collecting data needed to obtain registration for use in agricultural areas. We have designed a special spray rig that allows application of registered insecticides in such a manner to minimize impact on parasites.

The strong support from collaborator growers, the significant increase in insectary parasite production and field releases, the notable increase and spread of *Leptomastidea* following parasite releases under extremely hot temperatures, and the development of an effective ant control method, in total provide a strong basis for high expectations of effective control of VMB in the spring preceding harvest. That is also the period when the VMB parasites are at maximum effectiveness.

A. INTRODUCTION

This proposal continues the work of a diversified group interested in demonstrating an IPM system for the control of recently introduced vine mealybug (VMB) pests. This is being done by mass rearing two parasites (*Anagyrus pseudococci* and *Leptomastidea abnormis*) in a commercial insectary, releasing them in commercial vineyards, evaluating their establishment and effectiveness, finding a synergistic ant control method, and widely disseminating the results. This project is in its first year.

In June of 1994, a previously unencountered VMB pest was discovered in the Coachella Valley and other desert growing regions of Riverside County (Gill, 1994). This new pest represents a serious economic threat for California table grape cultivations because it feeds on the vines and produces copious quantities of honeydew (upon which mold develops rendering the fruit unmarketable). Additionally, it is a vector for two serious diseases: the grapevine corky bark virus and grapevine leafroll disease. Although the preferred hosts of *Planococcus ficus* (VMB) are apparently grape and fig, the genus has also been recorded as a pest of apple, avocado, banana, citrus, date palm, mango, pomegranate, and ornamentals (Cox, 1986). Potential thus exists for this pest to move to other crops and cause even more extensive economic damage. It has already spread to the San Joaquin Valley and threatens the entire grape industry in California.

Since 1994, insecticides have been the only significant management tactic used to attempt to prevent or reduce economic losses from mealybug damage. However, because of the mealybug's habit of congregating beneath bark and in other protected places, chemical controls are difficult or ineffective (Berlinger, 1977). Use of chemicals also upsets the existing natural balance, causing resurgence of the target pest and secondary pest outbreaks in grapes and other pests in adjacent crops such as alfalfa, citrus, dates, and ornamentals (González, 1998). In many crops, chemical applications rapidly result in resistant pest populations (especially spider mites) for which there are no known control measures.

Introducing parasites of VMB to the Coachella Valley should reduce the levels of overwintering mealybugs. These reduced levels of VMB will result in fewer insecticide applications thus allowing the native predators to increase in numbers and combine with introduced parasites to further lower mealybug numbers. This "field insectary" will maintain a needed level of beneficial insects in the vineyards while reducing environmental and worker risk.

In the Coachella Valley, substantial work has been completed by D. González and J. Klotz, UCR, CDFA, local cooperative extension personnel, and others on whose work we are building. Indigenous parasites attacking the vine mealybug in the Coachella Valley have been identified and assessed for impact. In a collaborative survey in 1994, 1995, 1996, 1997, and 1998 between University of California researchers, CDFA, and Riverside County personnel, low levels of native parasites of mealybugs were found.

An assessment of predator impact against VMB was made in 1998 in collaboration with CDFA personnel. Spiders appear to be the principal predators of VMB followed by green and brown lacewings and possibly coccinellids. However, there remains a definite lack of effectiveness by native natural enemies, by themselves, in the Coachella Valley.

Dr. Gonzalez completed field cage and open field evaluation studies on imported parasites over summers in 1996-1998 in vineyards in the Coachella Valley. The *A. pseudococci* from Spain and the *Leptomastidea* from Israel provided exceptionally outstanding results over the past two years and significantly better results than the *Anagyrus* indigenous to the Coachella Valley. Data obtained by D. Gonzalez in parasite evaluation trials in 1998 showed that harvest yields in pesticide-untreated plots were comparable or greater than yields in adjacent commercial vines receiving two applications of Methomyl. Movement of parasites has been confirmed from release to non-release areas with far greater numbers in release areas, based on data from yellow sticky traps.

Also, in preliminary trials, ants were found interfering with parasitization of mealybugs. Our preliminary results are supported by earlier reports (Nixon, 1951; Phillips & Sherk, 1991)) that ants interfere significantly with parasite impact on mealybugs. The most common ant pest in the Coachella Valley vineyards is a field ant, *Formica perpilosa* (Shorey and Neja, unpubl. data). This species thrives in the irrigated desert conditions characteristic of this region, and nests in large colonies at the base of the grapevines, where it is in close proximity to its major source of honeydew, the Vine Mealybug. *F. perpilosa* is a very active and aggressive ant (Wheeler and Wheeler, 1986). The other common pest in Coachella vineyards that tends and protects mealybug species is the southern fire ant, *Solenopsis xyloni* (Shorey, unpubl. data). Several materials and various techniques are being tested for ant control (Klotz et al, 1998; Reiersen et al, 1998; Shorey et al, 1992).

In a number of other countries, VMB populations are biologically controlled by several parasites (Rosen & Rossler, 1996; Berlinger, 1973a, 1973b, 1977; Myartseva, 1984; Myartseva & Nyazov, 1986; Cox, 1986, Triapitzyn, 1989). Therefore, work is being done to introduce exotic parasites to areas where mealybugs are unchecked.

B. MATERIALS AND METHODS

The goal is to develop an environmentally safe management program for a new and economically devastating pest of grapes. **The objectives are:** (1) to establish a functional IPM Innovator Program using guidelines provided by the DPR and CalEPA. This IPM Innovator Group is responsible for disseminating interim findings and final results of this project for implementation industry-wide. (2) to rear and mass release two species of mealybug parasites on a multi-farm scale and (3) to assess the effectiveness of the parasites against VMB and evaluate the status of colonization and/or augmentation success. Part of the evaluation also includes the impact of ants on parasite effectiveness.

OBJECTIVE 1: The IPM grape group is in place and operational because of the common interest in solving the VMB problem and the constant outreach and communication efforts of the core group. Following commitment of initial funding from DPR and other sources, the project got underway in April 1999. Acreage was set aside and modified to comply with the experimental requirements. A meeting was held June 1999, to plan this year's activities, identify each individual's role in this project, and to discuss primary concerns. Those in attendance included: Vincent Bianco, Anthony Farms (grower); David Fenn, Sun World (grower, PCA);

Dan Gonzalez, UCR (research); Harry Griffiths, FAR (insectary); Efrain Guzman, FAR (insectary technician); Charles Hunter, DPR (grant supervisor); Ross Jones, CTGC; John Klotz, UCR (ant control); Sharon Lasley, FAR (administration); Rudy Neja, Cooperative Extension; Revae Reynolds, Sun World (recording secretary); Vladimer Tudor, Tudor Ranches (grower).

A second and third meeting to review progress and to promote interim findings were held in October 1999 and in February 2000. Those invited to attend included those invited to the June meeting, as well as representatives from the Riverside County Agricultural Commissioner's office, additional grape growers, and local Pest Control Advisors.

At the completion of the first year of the project after harvest data are available, interim findings will be presented to the following publications and organizations: American Vineyard magazine, Grape Grower magazine, California Grower magazine, the California Table Grape Commission, the Riverside County Extension office, the California Desert Grape Administrative Committee, and local government agencies. Eddie Walker of Peter Rabbit Farms is chair of the Innovator Group and is coordinating the information/dissemination activities.

OBJECTIVE 2: The rearing work is being done at Foothill Ag Research, Inc. (FAR), in Corona, California. Since insulated trailers provide an excellent environment for producing insects, two 8' x 40' insulated trailers were purchased and equipped with electricity, shelving, air conditioning, heat pumps, lights, and plugs. The rearing procedure being used begins with establishing a host material on which to raise VMB. Once the VMB population is established, the parasites are introduced. As the parasite population grows and thrives, it is being harvested and released into the vineyards. The following food sources for rearing VMB were tested: banana squash, butternut squash, kabocha squash, and potato sprouts. Eight different soils (and soil compounds) and two types of containers (nursery flats and Rubbermaid plastic containers) were tested to find the best materials for sprouting potatoes. Some potatoes were treated with gibberellic acid and others were not.

The mealybug's life cycle involves a number of stages. In order to insure discreet instars of mealybug a crawler rack was developed for the production of vine mealybug crawlers. The rack was open and it was discovered that scymus ladybird beetle and the brown lacewing had seriously contaminated the culture. Having anticipated the possibility of contamination, a small back-up clean culture of mealybug was available. This enabled the insectary to begin production of a new mealybug culture without a complete loss. To reduce the possibility of future contamination, the following procedural changes were implemented: (1) To insure clean VMB crawlers, an enclosed crawler rack was developed (previously an open crawler rack was used). Also, the first rack design produced resulted in the production of various stages of the VMB, and the parasites did not parasitize all the VMB. Now, each rack will produce a uniform stage of VMB crawlers. The importance of a uniform culture is that *L. abnormis* attack the 1st, 2nd, and 3rd instars of the mealybug, while the *A. pseudococci* attack the 4th and 5th instars. (2) Three rooms for crawler production are being used; one is producing, one will start producing as soon as the first is finished and the third is clean. This is being done in a six week cycle. (3) Fourteen enclosed cabinets were placed in the mealybug rearing room, instead of a rearing room with open shelves. The idea being that if contamination occurs, it will be confined to one cabinet. (4) If any contamination does occur there is a back-up system that enables the insectary to

decontaminate the mealybug cabinets every four months. (5) There have been industrial size fans installed in all the production rooms (crawler, mealybug and parasite). These fans are turned on before the doors are open so that any contamination is blown away from the opening.

Release of parasites is by FAR personnel in the Coachella Valley test plots described below. In Year 1, the first releases were begun approximately three months after funding became available, and they continued through October. Beginning in Year 2, equal numbers of parasites are being released in each of 4 fields weekly from February through November. Maximum numbers of parasites will be reared from funding available. With funding requested, we will maintain current levels of parasite rearing and anticipate releasing 200,000 parasites weekly (100,000 *L. abnormis* and 100,000 *A. pseudococci*). This amount may be increased if we obtain additional funds to add additional rearing rooms. J. Barcinas will coordinate all release activities with growers and with D. Gonzalez and J. Klotz.

OBJECTIVE 3: Evaluation is conducted by University of California personnel. The experimental design is a randomized complete block with five treatments each in 4 replications (4 farms, see Fig. 1). Each of 4 growers (members of the Coachella Valley IPM Innovator Group) is providing approximately 6 acres untreated with chemicals (except for ant control through skirt treatments) for 4 treatments: (a) parasite release plus ant control, (b) parasite release, no ant control, (c) completely untreated (no ant control, no parasite release), (d) ant control only (no parasite release), plus a 5th treatment, (e) grower commercial pest control treatment (same treatments on all 4 farms). Samples are taken only from the center third of each plot. The outer 1/3 on each side of each plot serves as a buffer zone between treatments. Plots are located on the up-wind edge of all farms not adjacent to other vineyards. This minimizes insecticide drift, which readily kills parasites and predators.

Baseline Data: In 1999 and in 2000, pre-treatment VMB, parasite, and ant data were collected for a minimum of 1 week in each of the 4 vineyards.

Chemical treatments (skirt applications) against ants are applied in one of the two parasite release plots and in one of the two untreated (except for ants) control plots (Fig 1). We are using a registered material, Lorsban, for ant control and applying it with a modified sprayer we designed to minimize impact against parasites and predators (Fig. 2).

Evaluation of impact from treatments on mealybugs and yields is based on sampling techniques developed over the past 3 years by D. Gonzalez, the late H. Shorey (Univ. Calif.), J. Ball, and K. Godfrey (CDFA). Evaluation samples are taken every 2 weeks at each farm by D. Gonzalez, a technician from UCR, and two field assistants. Samples are staggered allowing sampling of 2 farms on odd-numbered weeks and 2 farms on even-numbered weeks. Evaluations are based on the following:

a) Parasite numbers are assessed every 2 weeks on each farm beginning one week after first release from February through November by placing 18 yellow sticky traps through the center third of the plots where parasites are released. Traps are left in the field for 2 weeks, and returned to the lab for identification and counts of parasites, predators, and mealybugs. Data from our trials and from J. Ball and K. Godfrey (CDFA) trials in 1998 showed these traps as

reliable as 2 other methods tested. Yellow traps have a great advantage in requiring a relatively short processing time thus allowing more samples to be collected. Similar samples are taken from the untreated control plots and from the commercial treatment plots.

b) Damage from mealybugs and mealybug numbers are estimated with visual observations in time-controlled samples from February until harvest date. The relatively short time needed to take each sample allows a greater number of samples with equal or greater sampling efficiency than other methods tested by D. Gonzalez, H. Shorey, J. Ball and K. Godfrey in 1998. We have three samplers taking a total of 18 samples per each of 5 treatments every 2 weeks. Data recorded includes frequency and intensity (size) of honeydew on trunks and vines, scored from 1 to 10. In the second portion of the sample, numbers of ants, and mealybugs are recorded

c) Estimates for ant abundance are taken from pitfall traps and visual observations. The pitfall traps sample ants on the ground, and the visual counts sample ants in the vines. There are 16 pitfall traps (4 in each of 4 quadrants) per plot. Samples and visual observations are taken bimonthly.

d) Yields will be recorded in boxes/acre (18-lb. equivalents) from each of the 5 treatment plots. In samples from ant control and no-ant control areas, we will record yield from both fruit-washed and unwashed for honeydew removal. Fruit wash is done directly in the field by dipping fruit with honeydew into 5-gal buckets of water and setting them aside to dry. These can be packed into separate boxes for recording boxes/acre of washed fruit.

Analyses of variance will be used to test for significant differences among treatments from **(I)** yellow trap data: (i) numbers of each parasite species released, (ii) numbers of each indigenous predator species, from **(II)** damage estimates (visual counts): (i) honeydew levels, (ii) numbers of mealybugs, and (iii) numbers and species of ants; and from **(III)** crop yields among the 5 treatments. Correlation and regression analyses will be used to compare crop yields vs. damage estimates and mealybug numbers; parasite species numbers in ant control vs. no ant control areas; and parasite and predator numbers (yellow traps) vs. damage and mealybug numbers (visual samples). Cost effectiveness of treatments will be based on fruit yield in boxes/acre vs. cost/box compared to commercially produced acreage. D. Gonzalez is supervising and participating in all activities dealing with experimental plot layout, sampling for evaluations, and analyses of data. He is coordinating all activities with D. Fenn, J. Barcinas, and J. Klotz.

The attached timetable indicates completion dates for each objective. Objectives 2 and 3 began in February and will continue through November. A minimum of two full seasons will be needed to estimate results from seasonal and between year's variation, for Objectives 2 and 3. Data are not collected in December – January because cold temperatures greatly reduce parasite, VMB and ant activity.

RESULTS:

April (funding date) to December 1999:

OBJECTIVE 1. Excellent cooperation exists among the collaborator growers as well as representatives of CDGAC, UCR, and the insectary in establishing a functional IPM Innovator Program using guidelines provided by the Department of Pesticide Regulation and the California Environmental Protection Agency. Four farms have been designated as the demonstration sites. Each site contains all elements required. Interested parties are being included in update meetings and in plans to promote the progress and results of this study. Two public meeting were held in 1999 to present progress reports (described earlier in materials and methods).

OBJECTIVE 2. All start-up activities were completed in the first two months including purchasing equipment, outfitting rearing trailers, identifying host materials for rearing insects, and identifying test plots. Evaluation of insectory methodologies has already resulted in improved technology for more cost effective and productive rearing of mealybugs and parasites, as discussed in Materials and Methods above.

Much effort was expended designing the most efficient and effective method of sprouting potatoes as a host material for the VMB. Of the 8 different soil types tested, sprouts were approximately 100% longer on the best soil as opposed to the least effective soil. The best soil produced about 5 times more sprouts per tray than the least effective soil. Of the two types of containers tested, the nursery flat sprouts had growth that varied by as much as 50%. In the Rubbermaid plastic containers, the variables were as little as 10%. Sprouts treated with gibberellic acid created considerably more "witches broom" sprouting while those not treated with gib grew more elongated sprouts that are preferable for hosting VMB. Unfortunately, the potato sprouts experienced contamination problems, with other insects damaging the culture, and the VMB produced were not uniform.

The following food sources for the VMB provided the following results: 1) Banana Squash was infested with VMB infested potato sprouts or mealybug crawlers. This squash is accepting either method for infesting. 2) Potato Sprouts were infested the same as #1 and are having similar acceptance as #1. 3) Butternut Squash does not accept crawlers well. They tend to move to the stylar end of the squash. When potato sprouts are used to infest, they tend to cover the squash uniformly. 4) Kabocha Squash: mealybug crawlers die on Kabocha. When potato sprouts are used, the mature VMB move to the squash and survive. This squash is producing relatively poor numbers of VMB, even though USDA literature states that the kabocha is an excellent host for VMB. Infesting the food sources with VMB crawlers produces a more uniform culture.

The food sources produced the following amounts of VMB per square inch: Banana Squash, 89 VMB; Butternut Squash, 89 VMB; Potato Sprouts, 118 VMB per 1 linear inch of sprout. Overall, even though the potato sprouts produced slightly more VMB, the culture proved to be time-consuming, inefficient, and costly. As a result, future rearing will take place on Banana or Butternut squash. While the insectary is still experimenting with the quality of the squash the VMB will infest, the VMB prefer the softer, immature, fresh squash.

Production of parasites will be enhanced next season also for the following reasons: (a) a source of fresh squash has been located (VMB require fresh squash). (b) Optimal VMB ages have been determined for each parasite: 10-12 day old VMB are superior host material for *Leptomastidea*; but 22-24 day old VMB are the optimal host material for *Anagyrus*. (c) As a result of production changes noted above the number of parasites available for sting has doubled. At the beginning of this project production was approximately 500 parasites per squash. The present production level is 2500 parasites (both species) per squash. (d) Contamination is greatly reduced and isolated because of the double enclosure of all rearing units.

With funding available in year 1, in 1999 approximately 175,000 to 245,000 parasites were produced weekly at full production. Numbers of parasites produced in 1999 are given in Table 1a. These numbers represent a highly significant increase in production of both of these parasites, much greater than any production records reported previously. Releases were scheduled to begin in early August. However, due to the success of the production of both parasites, releases were started on July 30, 1999. Release of parasites is done by FAR personnel in the Coachella test plots. Approximately 50-75% of the weekly production is being released weekly in the Coachella Valley, divided into 4 vineyards, as per the original experimental design. In 1999 we released an average of approximately 43,000 and 67,000 *Anagyrus pseudococchi* and *Leptomastidea abnormis*, respectively, per week from July 30 to October 8, 1999 (Table 1a).

OBJECTIVE 3.

Pre-treatment Data (prior to parasite releases or ant control)

Objectives have been met with respect to obtaining pre-treatment data (prior to parasite release) which provides a benchmark to compare with data obtained after the parasite releases. There are great differences in numbers of "native" or pre-existing parasites among all fields. There were also great differences in numbers of parasites recovered from the 2 species, with greater numbers of *Anagyrus* recovered than numbers of *Leptomastidea* (both findings on Table 2).

Data on VMB numbers were either zeros (two fields) or in very low numbers (two fields). Data on honeydew blotches on trunks and vines were also in relatively low incidence, although more readily detectable than live VMB. In general, the levels of honeydew blotches corresponded with the levels of live VMB. That is, honeydew blotches were notably higher in locations where VMB were found. Very low incidence (or none) of honeydew blotches were found in fields where there were zero live VMB.

Pitfall traps and visual time searches provided the best methods for assessing ant activity on the ground and in the vines. In visual observations, numbers of ants were also most closely correlated with sites where live VMB were found. Lowest (nearly zero) incidences of ants were from sites where there was zero live VMB and low incidence of honeydew blotches. In the absence of live VMB, ants were more abundant in areas where there was greater incidence of honeydew blotches.

Samples after parasite releases

The numbers of the VMB parasite *Leptomastidea* increased significantly in all 4 vineyards, most notably in parasite release areas (Table 4). *Leptomastidea* were recovered in 10 times greater numbers after releases compared with numbers prior to releases. *Leptomastidea* also increased in numbers as the season progressed. High levels of *Leptomastidea* continued up to more than two months after the last parasite releases on 8 October (Table 4). These results show that *Leptomastidea* are surviving and reproducing in the vineyards despite high day temperatures during August-October, and cold nights in November. The increase in numbers in non-release areas also indicates significant movement of these parasites from release to non-release areas. (Table 4).

We believe that the relatively low numbers of the VMB parasite *Anagyrus* recovered from release versus non-release areas in 1999 reflects the unusually high temperatures in the Coachella Valley from August through October. High temperatures are known to be detrimental to *Anagyrus*. However, in results from previous years, numbers of *Anagyrus* were equal to or better than those of *Leptomastidea*. Those results were taken from February through May when temperatures are relatively mild in the Coachella Valley.

Data collected in 1999 in our samples for live VMB, honeydew blotches, and ants were similar to those reported above under pre-treatment results. In samples taken approximately 2 and 4 weeks after the Lorsban ant treatment we found significant reductions in numbers of ants from treated vs untreated plots (Table 3). There were no significant reductions in numbers of parasites (*Anagyrus* or *Leptomastidea*) from treated vs untreated plots (Table 5).

A close relationship between VMB and ant activity has been documented, supporting the expectation that effective ant control will enhance parasite impact against VMB.

January to March 2000:

Objective 1: A field day was held on 17 March at Tudor ranch (one of our cooperators) to describe the VMB biological control program in the Coachella Valley. We presented objectives results and future plans. Representative of all personnel involved in this project were present and participated in the presentation (FAR Insectary, UCR research and extension, and all grower collaborators plus C. Hunter DPR, and J. Schrader from Riverside Co. Agric Com., PCA's from several companies, and several growers.

Objective 2: Modifications and improvements were continued in testing plant materials for rearing VMB and refinement of equipment to further reduce contamination of cultures.

Production and releases of *Anagyrus* is proceeding very well as reflected in weekly releases made beginning the first week of February (Table 1b). Except for the week of 4 February, over 192,000 *Anagyrus* have been released weekly over 7 weeks. The over-all average is more than 288,000 *Anagyrus* per week over the last 7 weeks.

Full scale releases of *Leptomastidea* have been variable. Regular full scale production of this parasite has been delayed because of recurring problems with equipment regulating relative

humidity. In 4 separate weeks when there were no humidity problems, more than 96,000 *Leptomastidea* were released each of those 4 weeks (Table 1b).

By the end of March we expect to be releasing weekly a minimum of 100,000 *Anagyrus* and 100,000 *Leptomastidea* in the Coachella Valley.

Objective 3: Two applications of Lorsban (on in February and one March) were made for ant control in designated plots on all 4 vineyards.

Low or 0 numbers of ants are being found in visual counts of ants in ant-treated plots. Significantly greater numbers of ants are being counted in non-treated plots.

VMB are beginning to appear and increase in numbers although levels are still relatively low. From counts in several fields there is a trend indicating notably higher levels of VMB in fields where commercial treatments were applied last year, and where we have not released parasites nor applied materials for ant control. Lowest numbers of VMB tend to be in plots treated with Lorsban for ants and/or where parasites were released.

Numbers of male VMB collected from yellow cards are closely correlated with the numbers of VMB in the 4 fields samples.

Numbers of parasites have been recorded on yellow sticky cards from all plots beginning the last week in January. Data are presently available only for pre-release collections (Table 6). Other cards are being counted and processed.

Data from pre-release samples show moderate levels of *Anagyrus* indicating reasonable survival over winter, and movement of the surviving parasites throughout fields and plots prior to releases this year. The data are strongly biased in favor of males.

Leptomastidea were recovered from pre-release samples in low numbers suggesting poor survival of winter conditions and/or emergence perhaps at a later date when temperatures are higher.

DISCUSSION:

Because of the funding date, work on Objectives 2 and 3 began after harvest and toward the end of the growing season during the first year of funding. Nevertheless, the system for production of parasites has been greatly improved and the results are that up to more than 25 times as many parasites are being produced as we had anticipated from previously using the traditional rearing methods. The lower cost of producing greater numbers of parasites with reduced contamination provides a stronger potential for biological control colonization and/or augmentation programs (Luck et al, 1992).

Results are incomplete for several reasons. After harvest (July), VMB retreat to refuge areas under the bark or to the roots. They become extremely difficult to sample under these conditions. Because of the low numbers of VMB after harvest the principal impact from our

studies will be demonstrated next spring when VMB, parasites, and ant activity is optimal. At the present stage, these preliminary results provide a strong basis for expecting significant impact from parasite releases against VMB in the spring when parasites are most effective. In addition to producing greater numbers of 2 types of parasites, we will also have effective reduction in ant activity without affecting parasite numbers to enhance the parasites' effectiveness. At the end of fiscal Year 1 (May 2000) we will have preliminary data on the impact from mass release of parasites on mealybug populations during the season, on over-wintering populations, and on yields. A minimum of two full seasons will be needed to estimate results from seasonal and between year's variation, for Objectives 2 and 3.

For the purpose of this report, it should be noted that funds from several sources were awarded for the rearing and releasing of the parasites, for the ant studies, and for evaluations. Additional funding to that provided by DPR was provided by the California Desert Grape Administrative Committee (CDGAC), the California Grape Commission and the Cooperative State Research, Education, and Extension Service of the USDA.

SUMMARY AND CONCLUSION:

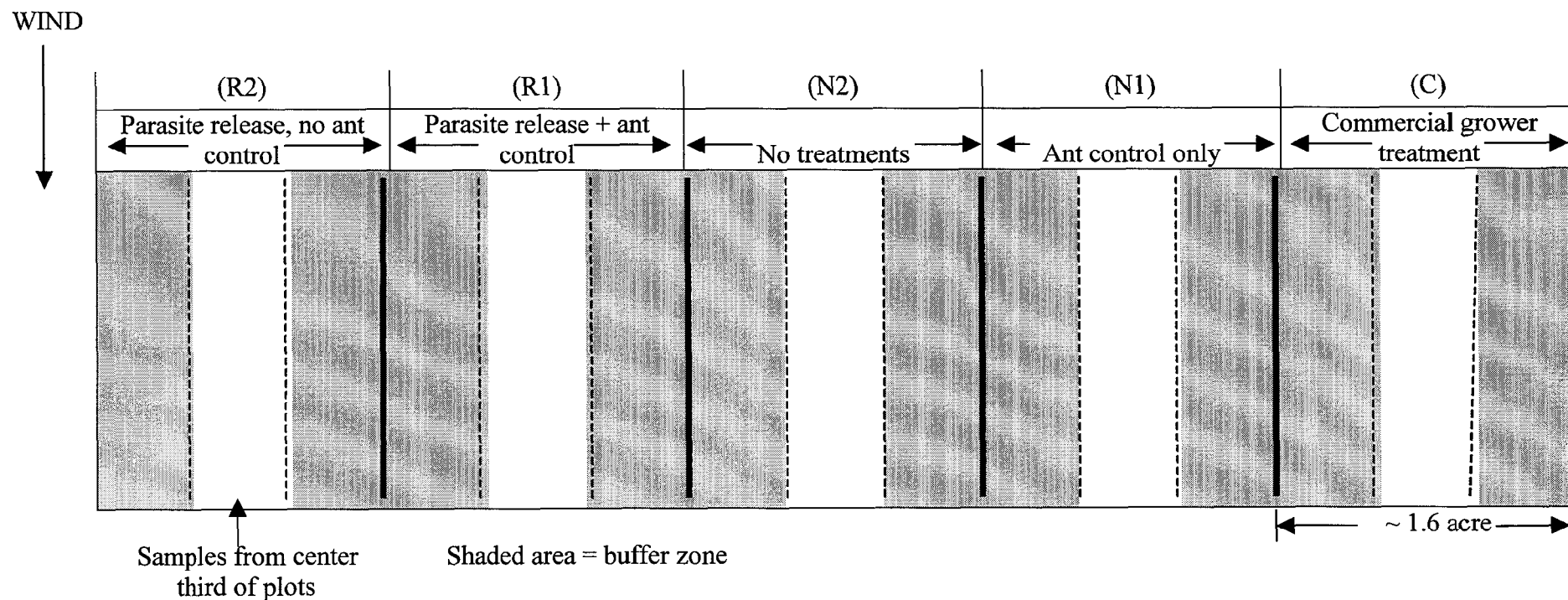
Significant progress has been made in each of the 3 objectives in this proposal. In objective 1, a group of collaborators has provided all resources needed to establish viable study/demonstration sites. In objective 2, insectary parasite production techniques have been modified resulting in a great increase in numbers of parasites produced and released, also with significant reduction in the potential for contamination of insectary rearing colonies. The significant increase in insectary production of parasites greatly enhances the potential to reduce VMB damage with biological control colonization and/or augmentation programs. In objective 3, we have demonstrated significant increases in *Leptomastidea* recovered following parasite releases under extremely hot temperatures. We have documented a close relationship between VMB and ant activity, supporting our expectation that effective ant control will enhance parasite impact against VMB. We have tested several baits for ant control and are collecting data needed to obtain registration for use in agricultural areas. We have designed a special spray rig that allows application of registered insecticides in such a manner to minimize impact on parasites.

The strong support from collaborator growers, the significant increase in insectary parasite production and field releases, the notable increase in *Leptomastidea* following parasite releases under extremely hot temperatures, and the development of an effective ant control method, in total provide a strong basis for high expectations of effective control of VMB in the spring preceding harvest next year. That is also the period when the VMB parasites are at maximum effectiveness.

LITERATURE CITED

- Berlinger, M. J. (1973a).** Effect of relative humidity and temperature on the survival of *Clausenia josefi* (Hymenoptera: Encyrtidae). *Entomologia Experimental et Applicata* 16: 373-79.
- Berlinger, M. J. (1973b).** Biological studies of *Clausenia josefi* (hymenoptera: Encyrtidae), a parasite of *Planococcus vitis*. *Entomophaga*. 18 (3): 297-86.
- Berlinger, M.J. (1977).** The Mediterranean vine mealybug and its natural enemies in southern Israel. *Pyhytoparasitca*
- Cox, J.M. (1986).** Planococcine mealybugs of economic importance from the Mediterranean Basin and their distinction from a new African genus (Hemiptera: Pseudocidae). *Bull. Ent. Res.* 76: 481-489.
- Gill, R.J. (1994).** The vine mealybug (*Planococcus ficus*): a mealybug new to California. USDA Report/Letter to Eldon Reeves (Riv.Co. Aa. Commissioner's Office).
- González, D. 1998.** Naturally-occurring biological control, the most important component in crop protection programs. P. 183-186. *In*: Calif. Conf. Biol. Control Innovation in Biol. Control Research (M. Hoddle ed.) Berkeley, Calif.
- Klotz, J. H., Greenberg, L. and E. C. Venn. 1998.** Liquid boric acid bait for control of the Argentine ant (Hymenoptera: Formicidae). *J. Econ. Entomol.* 91:910-914.
- Luck, R., J. Morse, P. Haney, H. Gjiriffiths, J. Barcinas, T. Roberts, E. Grafton-Cardwell, and N. O'Connell. 1992.** Citrus IPM – It Works. *Calif. Grower* 16 (4): 25-27.
- Myartseva, S.N. (1984).** Natural enemies of the family Encyrtidae (Hymenoptera, Chalcidoidea) in turkmenistan and scientific bases for their use in plant protection. *Izvestiya akademii Nauk Turkmenskoi SSR, Biologicvheskikh Nauk* (No. 2): 23-30.
- Myartseva, S. N. and O. D. Nyazov. (1986).** Vine mealybug and its natural enemies in Turkmenistan. (V. A. Triapitzin, Ed.) Ashkhabad: Ylym Publ. 92 pp.
- Nixon, G.E.J. (1951).** The association of the ants with aphids and coccids. *Commun. Inst. Ent.*, London.
- Phillips, P. and C. Sherk. 1991.** To control mealybugs, stop honeydew seeking ants. *Calif. Agric.* 45: 26-28.
- Reierson, D. A., Rust, M. K., and J. Hampton-Beesley. 1998.** Monitoring with sugar water to determine the efficacy of treatments to control Argentine ants, *Linephithema humile* (Mayr). *In* Proceedings of the National Conference on Urban Entomology, San Diego, Calif.
- Rosen, D. and Y. Rossler. (1966).** Studies of an Israel strain of *Anagyrus pseudococci* (Girault) Hymenoptera: Encyrtidae). I. Morphology of the adults and development stages. *Entomophaga* 11: 269-77.
- Shorey, H.H., L.K. Gaston, R.G. Gerber, P.P. Phillips, and D.L. Wood. (1992).** Disruption of foraging by Argentine ants, *Iridomyrmex humilis* (Mayr) (Hymenoptera: Formicidae), in citrus trees through the use of semichemicals and related chemicals. *J. Chem. Ecol.* 18(11): 2131-142.
- Triapitzyn, V. A. (1989).** Parasitic Hymenoptera of the Family Encyrtidae of the Palaearctics Leningrad, Nauka, Leningrad Division. 488pp.
- Wheeler, G. C. and Wheeler, J. N. (1986).** The ants of Nevada. A publication of Natural History Museum of Los Angeles Country, Allen Press, Lawrence, KS.

Fig. 1. Schematic Diagram of Experimental Design (field-plot arrangement)



Plots arranged randomly in each field

No insecticide applications up-wind

1 = ant control

2 = no ant control

R = parasite release

N = no parasite release

C = commercial applications

Fig. 2. Spray-rig for Lorsban to control *F. perpilosa* in grape vineyards



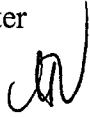


Riverside

DEPARTMENT OF ENTOMOLOGY
Interoffice Memorandum

Date: March 30, 2000

To: Charles Hunter

From: Helen Vega 
Academic Assistant

Subject: Final Report Contract #98-0262

Enclosed here is a copy of the subject final report. The report is copied to diskette in 6.0 for MS Word, however, the report and attachments were created in Word 2000, the tables and figures may lose their format on the diskette as a result of saving them as 6.0.

TITLE PAGE:

PEST MANAGEMENT GRANTS FINAL REPORT

Contract Number: 98-0262

Contract Title: Mass Releases of Natural Enemies of Vine Mealybug

Principal Investigators: Harry Griffiths / Joe Barcinas

Contractor Organization: Foothill Agricultural Research (FAR)

Date: March 31, 2000

Prepared for California Department of Pesticide Regulation

Disclaimer

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Department of Pesticide Regulation. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as actual or implied endorsement of such products.

Acknowledgements

This report was submitted in fulfillment of DPR contract No. 98-0262 Mass Release of Natural Enemies of Vine Mealybug by FAR Inc., under the partial sponsorship of the California Department of Pesticide Regulation. Work was completed as of March 2000.

We are grateful to growers in the Coachella Valley especially V. Tudor from Tudor Ranch, E. Walker from Peter Rabbit Farms, D. Fenn from Sunworld Inc., and V. Bianco from Anthony Vineyards, for their cooperation and support including providing all land and facilities used in this study. We are also grateful for partial financial support to the California Desert Grape Administrative Committee.

Ant control and ant sampling were conducted by J. Klotz and K. Kido, University of California, Riverside. Funding for these activities was by other agencies. DPR funds were not used in these activities.

Evaluation of parasite impact on VMB and ant impact on parasites was conducted by D. González, T. Dinoff, and K. McGiffen, University of California, Riverside. Funding for these activities was by other agencies. DPR funds were not used in these activities.

Contract Title: Mass Releases of Natural Enemies of Vine Mealybug

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ABSTRACT:

This is an eleven-month report (effective April 1999) on an effort by Coachella Valley table grape growers, a commercial insectary, University of California, Riverside researchers and extension personnel to implement a long-term reduced-risk pest management system to control recently introduced vine mealybug (VMB) pests and promoting this approach to all growers and interested parties.

Progress includes: (1) Collaborators have provided all resources needed to establish and operate viable study/validation sites. (2) Insectary parasite production techniques have been modified resulting in up to a 25 times increase in numbers of parasites produced than in previous years, also with significant reduction in contamination of rearing colonies. The significant increase in production of parasites greatly enhances the potential to control VMB with biological control colonization and/or augmentation programs. (3) Significant increases in the VMB parasite *Leptomastidea* were found following parasite releases in spite of extremely hot temperatures. (4) We found a close relationship between VMB and ant activity, supporting the expectation that ant control will enhance parasite impact against VMB. (5) Using a modified ground rig, ants were controlled effectively for several weeks without affecting parasite numbers.

The progress reported above provide the basis for effective control of VMB in the spring preceding harvest.

EXECUTIVE SUMMARY

The vine mealybug (VMB) was first discovered in the Coachella Valley in Southern California in 1994. It has spread rapidly, causing severe economic damage to table grape vineyards in the region. More recently, it was discovered in the San Joaquin Valley. The University of California Cooperative Extension Viticulture Advisor for Kern County stated, "...at the present time, table grapes in the Coachella Valley are severely impacted, however the spread of this pest into the central valley now exposes over 600,000 acres of grapes to this problem"

Due to the lack of effective native parasites, a dramatic increase in the application of organophosphates (Chlorpyrifos, Diazinon, and Dimethoate) and Carbamates (Carbaryl and Methomyl) has been implemented to control the vine mealybug. Each of these pesticides is on the EPA's list of chemicals to be reviewed as required by the Food Quality Protection Act (FQPA) of 1996. Although the USDA and EPA have not listed table grapes as a commodity whose production depends heavily on the pesticides included on the priority list, table grape production will be adversely impacted if the above listed materials are not available. Regardless of the status of these chemicals, a biological, sustainable solution to the devastating VMB is necessary. In essence, there is no effective control for VMB currently available because even pesticides are only marginally effective due to VMB's living under the bark until fruit formation and the protection of VMB by ants.

The objectives for this proposal are to bring together a voluntary effort by Coachella Valley table grape growers, a commercial insectary, University of California, Riverside (UCR) researchers, and extension personnel for the purpose of implementing a long-term, reduced-risk pest management system for the control of vine mealybug. This will be done by mass-rearing parasites in a commercial insectary, releasing them in commercial vineyards, evaluating their establishment and effectiveness, and widely disseminating the results to encourage others to adopt this IPM approach.

The IPM grape group is well established and functioning effectively. All land and facilities for the program have been provided by growers in the Coachella Valley. The experimental design consists of approximately 8 acres in each of 4 farms with 5 treatments on each farm: (1) no treatment of any kind (reference data); (2) ant control only; (3) ant control + parasite releases; (4) parasite releases only; and (5) grower commercial applications.

Rearing and releasing of parasites is proceeding exceptionally well, much better than expected. We expect production to exceed 100,000 of each parasite species per week, a figure much higher than ever reported previously, anywhere. Production has exceeded expectations because of improvement in food (host plant) provided to VMB, and significant improvement in rearing techniques and equipment, including special equipment to greatly reduce contamination. Releases in 1999 over an 8 week period exceeded an average of 67,000/wk *Anagyrus* and 43,000/wk *Leptomastidea*. In 2000 from February-March over a 7 week period releases of *Anagyrus* have exceeded an average of 288,000/wk and 54,800/wk *Leptomastidea*. We easily expect parasite releases of over 100,000/wk of each species during April-May the most critical period to prevent damage by VMB in the Coachella Valley.

Ant control and monitoring of ant species and numbers is producing better than expected results. Ants are being treated with Lorsban using a specially designed spray rig that effectively kills ants without affecting parasites. With pitfall traps and visual observations we have recorded greatly reduced ant numbers in treated areas, up to 4 weeks effective reduction on vines.

From yellow sticky trap data on evaluation of parasites impact on VMB and impact of ants on parasites we have found the following: parasites survive, increase in numbers, and spread to non-release areas, up to 2 months after being released, including periods of time when temperatures are extremely high (over 110°F). Ants are present in high numbers and with very aggressive behavior and are closely associated with VMB. In results from visual samples over 8 weeks this spring (February-March) we find a strong trend indicating lowest numbers of VMB where we have treated for ant control or where parasites have been released. Notably higher numbers of VMB are being found in the commercial plots where there have been no parasite releases or ant control.

In summary, significant progress has been made in each of the 3 objectives in this proposal. In objective 1, a group of collaborators has provided all resources needed to establish viable study/demonstration sites and outside interested people and organizations are involved in the progress of this study. In objective 2, insectary parasite production techniques have been modified resulting in a great increase in numbers of parasites produced and released, also with significant reduction in the potential for contamination of insectary rearing colonies. In objective 3, we have demonstrated significant increases in *Leptomastidea* recovered following parasite releases under extremely hot temperatures. We have documented a close relationship between VMB and ant activity, supporting our expectation that effective ant control will enhance parasite impact against VMB. We have tested several baits for ant control and are collecting data needed to obtain registration for use in agricultural areas. We have designed a special spray rig that allows application of registered insecticides in such a manner to minimize impact on parasites.

The strong support from collaborator growers, the significant increase in insectary parasite production and field releases, the notable increase and spread of *Leptomastidea* following parasite releases under extremely hot temperatures, and the development of an effective ant control method, in total provide a strong basis for high expectations of effective control of VMB in the spring preceding harvest. That is also the period when the VMB parasites are at maximum effectiveness.

A. INTRODUCTION

This proposal continues the work of a diversified group interested in demonstrating an IPM system for the control of recently introduced vine mealybug (VMB) pests. This is being done by mass rearing two parasites (*Anagyrus pseudococchi* and *Leptomastidea abnormis*) in a commercial insectary, releasing them in commercial vineyards, evaluating their establishment and effectiveness, finding a synergistic ant control method, and widely disseminating the results. This project is in its first year.

In June of 1994, a previously unencountered VMB pest was discovered in the Coachella Valley and other desert growing regions of Riverside County (Gill, 1994). This new pest represents a serious economic threat for California table grape cultivations because it feeds on the vines and produces copious quantities of honeydew (upon which mold develops rendering the fruit unmarketable). Additionally, it is a vector for two serious diseases: the grapevine corky bark virus and grapevine leafroll disease. Although the preferred hosts of *Planococcus ficus* (VMB) are apparently grape and fig, the genus has also been recorded as a pest of apple, avocado, banana, citrus, date palm, mango, pomegranate, and ornamentals (Cox, 1986). Potential thus exists for this pest to move to other crops and cause even more extensive economic damage. It has already spread to the San Joaquin Valley and threatens the entire grape industry in California.

Since 1994, insecticides have been the only significant management tactic used to attempt to prevent or reduce economic losses from mealybug damage. However, because of the mealybug's habit of congregating beneath bark and in other protected places, chemical controls are difficult or ineffective (Berlinger, 1977). Use of chemicals also upsets the existing natural balance, causing resurgence of the target pest and secondary pest outbreaks in grapes and other pests in adjacent crops such as alfalfa, citrus, dates, and ornamentals (González, 1998). In many crops, chemical applications rapidly result in resistant pest populations (especially spider mites) for which there are no known control measures.

Introducing parasites of VMB to the Coachella Valley should reduce the levels of overwintering mealybugs. These reduced levels of VMB will result in fewer insecticide applications thus allowing the native predators to increase in numbers and combine with introduced parasites to further lower mealybug numbers. This "field insectary" will maintain a needed level of beneficial insects in the vineyards while reducing environmental and worker risk.

In the Coachella Valley, substantial work has been completed by D. González and J. Klotz, UCR, CDFA, local cooperative extension personnel, and others on whose work we are building. Indigenous parasites attacking the vine mealybug in the Coachella Valley have been identified and assessed for impact. In a collaborative survey in 1994, 1995, 1996, 1997, and 1998 between University of California researchers, CDFA, and Riverside County personnel, low levels of native parasites of mealybugs were found.

An assessment of predator impact against VMB was made in 1998 in collaboration with CDFA personnel. Spiders appear to be the principal predators of VMB followed by green and brown lacewings and possibly coccinellids. However, there remains a definite lack of effectiveness by native natural enemies, by themselves, in the Coachella Valley.

Dr. Gonzalez completed field cage and open field evaluation studies on imported parasites over summers in 1996-1998 in vineyards in the Coachella Valley. The *A. pseudococci* from Spain and the *Leptomastidea* from Israel provided exceptionally outstanding results over the past two years and significantly better results than the *Anagyrus* indigenous to the Coachella Valley. Data obtained by D. Gonzalez in parasite evaluation trials in 1998 showed that harvest yields in pesticide-untreated plots were comparable or greater than yields in adjacent commercial vines receiving two applications of Methomyl. Movement of parasites has been confirmed from release to non-release areas with far greater numbers in release areas, based on data from yellow sticky traps.

Also, in preliminary trials, ants were found interfering with parasitization of mealybugs. Our preliminary results are supported by earlier reports (Nixon, 1951; Phillips & Sherk, 1991)) that ants interfere significantly with parasite impact on mealybugs. The most common ant pest in the Coachella Valley vineyards is a field ant, *Formica perpilosa* (Shorey and Neja, unpubl. data). This species thrives in the irrigated desert conditions characteristic of this region, and nests in large colonies at the base of the grapevines, where it is in close proximity to its major source of honeydew, the Vine Mealybug. *F. perpilosa* is a very active and aggressive ant (Wheeler and Wheeler, 1986). The other common pest in Coachella vineyards that tends and protects mealybug species is the southern fire ant, *Solenopsis xyloni* (Shorey, unpubl. data). Several materials and various techniques are being tested for ant control (Klotz et al, 1998; Reiersen et al, 1998; Shorey et al, 1992).

In a number of other countries, VMB populations are biologically controlled by several parasites (Rosen & Rossler, 1996; Berlinger, 1973a, 1973b, 1977; Myartseva, 1984; Myartseva & Nyazov, 1986; Cox, 1986, Triapitzyn, 1989). Therefore, work is being done to introduce exotic parasites to areas where mealybugs are unchecked.

B. MATERIALS AND METHODS

The goal is to develop an environmentally safe management program for a new and economically devastating pest of grapes. **The objectives are:** (1) to establish a functional IPM Innovator Program using guidelines provided by the DPR and CalEPA. This IPM Innovator Group is responsible for disseminating interim findings and final results of this project for implementation industry-wide. (2) to rear and mass release two species of mealybug parasites on a multi-farm scale and (3) to assess the effectiveness of the parasites against VMB and evaluate the status of colonization and/or augmentation success. Part of the evaluation also includes the impact of ants on parasite effectiveness.

OBJECTIVE 1: The IPM grape group is in place and operational because of the common interest in solving the VMB problem and the constant outreach and communication efforts of the core group. Following commitment of initial funding from DPR and other sources, the project got underway in April 1999. Acreage was set aside and modified to comply with the experimental requirements. A meeting was held June 1999, to plan this year's activities, identify each individual's role in this project, and to discuss primary concerns. Those in attendance included: Vincent Bianco, Anthony Farms (grower); David Fenn, Sun World (grower, PCA);

Dan Gonzalez, UCR (research); Harry Griffiths, FAR (insectary); Efrain Guzman, FAR (insectary technician); Charles Hunter, DPR (grant supervisor); Ross Jones, CTGC; John Klotz, UCR (ant control); Sharon Lasley, FAR (administration); Rudy Neja, Cooperative Extension; Reva Reynolds, Sun World (recording secretary); Vladimir Tudor, Tudor Ranches (grower).

A second and third meeting to review progress and to promote interim findings were held in October 1999 and in February 2000. Those invited to attend included those invited to the June meeting, as well as representatives from the Riverside County Agricultural Commissioner's office, additional grape growers, and local Pest Control Advisors.

At the completion of the first year of the project after harvest data are available, interim findings will be presented to the following publications and organizations: American Vineyard magazine, Grape Grower magazine, California Grower magazine, the California Table Grape Commission, the Riverside County Extension office, the California Desert Grape Administrative Committee, and local government agencies. Eddie Walker of Peter Rabbit Farms is chair of the Innovator Group and is coordinating the information/dissemination activities.

OBJECTIVE 2: The rearing work is being done at Foothill Ag Research, Inc. (FAR), in Corona, California. Since insulated trailers provide an excellent environment for producing insects, two 8' x 40' insulated trailers were purchased and equipped with electricity, shelving, air conditioning, heat pumps, lights, and plugs. The rearing procedure being used begins with establishing a host material on which to raise VMB. Once the VMB population is established, the parasites are introduced. As the parasite population grows and thrives, it is being harvested and released into the vineyards. The following food sources for rearing VMB were tested: banana squash, butternut squash, kabocha squash, and potato sprouts. Eight different soils (and soil compounds) and two types of containers (nursery flats and Rubbermaid plastic containers) were tested to find the best materials for sprouting potatoes. Some potatoes were treated with gibberellic acid and others were not.

The mealybug's life cycle involves a number of stages. In order to insure discreet instars of mealybug a crawler rack was developed for the production of vine mealybug crawlers. The rack was open and it was discovered that scymus ladybird beetle and the brown lacewing had seriously contaminated the culture. Having anticipated the possibility of contamination, a small back-up clean culture of mealybug was available. This enabled the insectary to begin production of a new mealybug culture without a complete loss. To reduce the possibility of future contamination, the following procedural changes were implemented: (1) To insure clean VMB crawlers, an enclosed crawler rack was developed (previously an open crawler rack was used). Also, the first rack design produced resulted in the production of various stages of the VMB, and the parasites did not parasitize all the VMB. Now, each rack will produce a uniform stage of VMB crawlers. The importance of a uniform culture is that *L. abnormis* attack the 1st, 2nd, and 3rd instars of the mealybug, while the *A. pseudococci* attack the 4th and 5th instars. (2) Three rooms for crawler production are being used; one is producing, one will start producing as soon as the first is finished and the third is clean. This is being done in a six week cycle. (3) Fourteen enclosed cabinets were placed in the mealybug rearing room, instead of a rearing room with open shelves. The idea being that if contamination occurs, it will be confined to one cabinet. (4) If any contamination does occur there is a back-up system that enables the insectary to

decontaminate the mealybug cabinets every four months. (5) There have been industrial size fans installed in all the production rooms (crawler, mealybug and parasite). These fans are turned on before the doors are open so that any contamination is blown away from the opening.

Release of parasites is by FAR personnel in the Coachella Valley test plots described below. In Year 1, the first releases were begun approximately three months after funding became available, and they continued through October. Beginning in Year 2, equal numbers of parasites are being released in each of 4 fields weekly from February through November. Maximum numbers of parasites will be reared from funding available. With funding requested, we will maintain current levels of parasite rearing and anticipate releasing 200,000 parasites weekly (100,000 *L. abnormis* and 100,000 *A. pseudococci*). This amount may be increased if we obtain additional funds to add additional rearing rooms. J. Barcinas will coordinate all release activities with growers and with D. Gonzalez and J. Klotz.

OBJECTIVE 3: Evaluation is conducted by University of California personnel. The experimental design is a randomized complete block with five treatments each in 4 replications (4 farms, see Fig. 1). Each of 4 growers (members of the Coachella Valley IPM Innovator Group) is providing approximately 6 acres untreated with chemicals (except for ant control through skirt treatments) for 4 treatments: (a) parasite release plus ant control, (b) parasite release, no ant control, (c) completely untreated (no ant control, no parasite release), (d) ant control only (no parasite release), plus a 5th treatment, (e) grower commercial pest control treatment (same treatments on all 4 farms). Samples are taken only from the center third of each plot. The outer 1/3 on each side of each plot serves as a buffer zone between treatments. Plots are located on the up-wind edge of all farms not adjacent to other vineyards. This minimizes insecticide drift, which readily kills parasites and predators.

Baseline Data: In 1999 and in 2000, pre-treatment VMB, parasite, and ant data were collected for a minimum of 1 week in each of the 4 vineyards.

Chemical treatments (skirt applications) against ants are applied in one of the two parasite release plots and in one of the two untreated (except for ants) control plots (Fig 1). We are using a registered material, Lorsban, for ant control and applying it with a modified sprayer we designed to minimize impact against parasites and predators (Fig. 2).

Evaluation of impact from treatments on mealybugs and yields is based on sampling techniques developed over the past 3 years by D. Gonzalez, the late H. Shorey (Univ. Calif.), J. Ball, and K. Godfrey (CDFA). Evaluation samples are taken every 2 weeks at each farm by D. Gonzalez, a technician from UCR, and two field assistants. Samples are staggered allowing sampling of 2 farms on odd-numbered weeks and 2 farms on even-numbered weeks. Evaluations are based on the following:

a) Parasite numbers are assessed every 2 weeks on each farm beginning one week after first release from February through November by placing 18 yellow sticky traps through the center third of the plots where parasites are released. Traps are left in the field for 2 weeks, and returned to the lab for identification and counts of parasites, predators, and mealybugs. Data from our trials and from J. Ball and K. Godfrey (CDFA) trials in 1998 showed these traps as

reliable as 2 other methods tested. Yellow traps have a great advantage in requiring a relatively short processing time thus allowing more samples to be collected. Similar samples are taken from the untreated control plots and from the commercial treatment plots.

b) Damage from mealybugs and mealybug numbers are estimated with visual observations in time-controlled samples from February until harvest date. The relatively short time needed to take each sample allows a greater number of samples with equal or greater sampling efficiency than other methods tested by D. Gonzalez, H. Shorey, J. Ball and K. Godfrey in 1998. We have three samplers taking a total of 18 samples per each of 5 treatments every 2 weeks. Data recorded includes frequency and intensity (size) of honeydew on trunks and vines, scored from 1 to 10. In the second portion of the sample, numbers of ants, and mealybugs are recorded

c) Estimates for ant abundance are taken from pitfall traps and visual observations. The pitfall traps sample ants on the ground, and the visual counts sample ants in the vines. There are 16 pitfall traps (4 in each of 4 quadrants) per plot. Samples and visual observations are taken bimonthly.

d) Yields will be recorded in boxes/acre (18-lb. equivalents) from each of the 5 treatment plots. In samples from ant control and no-ant control areas, we will record yield from both fruit-washed and unwashed for honeydew removal. Fruit wash is done directly in the field by dipping fruit with honeydew into 5-gal buckets of water and setting them aside to dry. These can be packed into separate boxes for recording boxes/acre of washed fruit.

Analyses of variance will be used to test for significant differences among treatments from (I) yellow trap data: (i) numbers of each parasite species released, (ii) numbers of each indigenous predator species, from (II) damage estimates (visual counts): (i) honeydew levels, (ii) numbers of mealybugs, and (iii) numbers and species of ants; and from (III) crop yields among the 5 treatments. Correlation and regression analyses will be used to compare crop yields vs. damage estimates and mealybug numbers; parasite species numbers in ant control vs. no ant control areas; and parasite and predator numbers (yellow traps) vs. damage and mealybug numbers (visual samples). Cost effectiveness of treatments will be based on fruit yield in boxes/acre vs. cost/box compared to commercially produced acreage. D. Gonzalez is supervising and participating in all activities dealing with experimental plot layout, sampling for evaluations, and analyses of data. He is coordinating all activities with D. Fenn, J. Barcinas, and J. Klotz.

The attached timetable indicates completion dates for each objective. Objectives 2 and 3 began in February and will continue through November. A minimum of two full seasons will be needed to estimate results from seasonal and between year's variation, for Objectives 2 and 3. Data are not collected in December – January because cold temperatures greatly reduce parasite, VMB and ant activity.

RESULTS:

April (funding date) to December 1999:

OBJECTIVE 1. Excellent cooperation exists among the collaborator growers as well as representatives of CDGAC, UCR, and the insectary in establishing a functional IPM Innovator Program using guidelines provided by the Department of Pesticide Regulation and the California Environmental Protection Agency. Four farms have been designated as the demonstration sites. Each site contains all elements required. Interested parties are being included in update meetings and in plans to promote the progress and results of this study. Two public meeting were held in 1999 to present progress reports (described earlier in materials and methods).

OBJECTIVE 2. All start-up activities were completed in the first two months including purchasing equipment, outfitting rearing trailers, identifying host materials for rearing insects, and identifying test plots. Evaluation of insectory methodologies has already resulted in improved technology for more cost effective and productive rearing of mealybugs and parasites, as discussed in Materials and Methods above.

Much effort was expended designing the most efficient and effective method of sprouting potatoes as a host material for the VMB. Of the 8 different soil types tested, sprouts were approximately 100% longer on the best soil as opposed to the least effective soil. The best soil produced about 5 times more sprouts per tray than the least effective soil. Of the two types of containers tested, the nursery flat sprouts had growth that varied by as much as 50%. In the Rubbermaid plastic containers, the variables were as little as 10%. Sprouts treated with gibberellic acid created considerably more "witches broom" sprouting while those not treated with gib grew more elongated sprouts that are preferable for hosting VMB. Unfortunately, the potato sprouts experienced contamination problems, with other insects damaging the culture, and the VMB produced were not uniform.

The following food sources for the VMB provided the following results: 1) Banana Squash was infested with VMB infested potato sprouts or mealybug crawlers. This squash is accepting either method for infesting. 2) Potato Sprouts were infested the same as #1 and are having similar acceptance as #1. 3) Butternut Squash does not accept crawlers well. They tend to move to the stylar end of the squash. When potato sprouts are used to infest, they tend to cover the squash uniformly. 4) Kabocha Squash: mealybug crawlers die on Kabocha. When potato sprouts are used, the mature VMB move to the squash and survive. This squash is producing relatively poor numbers of VMB, even though USDA literature states that the kabocha is an excellent host for VMB. Infesting the food sources with VMB crawlers produces a more uniform culture.

The food sources produced the following amounts of VMB per square inch: Banana Squash, 89 VMB; Butternut Squash, 89 VMB; Potato Sprouts, 118 VMB per 1 linear inch of sprout. Overall, even though the potato sprouts produced slightly more VMB, the culture proved to be time-consuming, inefficient, and costly. As a result, future rearing will take place on Banana or Butternut squash. While the insectary is still experimenting with the quality of the squash the VMB will infest, the VMB prefer the softer, immature, fresh squash.

Production of parasites will be enhanced next season also for the following reasons: (a) a source of fresh squash has been located (VMB require fresh squash). (b) Optimal VMB ages have been determined for each parasite: 10-12 day old VMB are superior host material for *Leptomastidea*; but 22-24 day old VMB are the optimal host material for *Anagyrus*. (c) As a result of production changes noted above the number of parasites available for sting has doubled. At the beginning of this project production was approximately 500 parasites per squash. The present production level is 2500 parasites (both species) per squash. (d) Contamination is greatly reduced and isolated because of the double enclosure of all rearing units.

With funding available in year 1, in 1999 approximately 175,000 to 245,000 parasites were produced weekly at full production. Numbers of parasites produced in 1999 are given in Table 1a. These numbers represent a highly significant increase in production of both of these parasites, much greater than any production records reported previously. Releases were scheduled to begin in early August. However, due to the success of the production of both parasites, releases were started on July 30, 1999. Release of parasites is done by FAR personnel in the Coachella test plots. Approximately 50-75% of the weekly production is being released weekly in the Coachella Valley, divided into 4 vineyards, as per the original experimental design. In 1999 we released an average of approximately 43,000 and 67,000 *Anagyrus pseudococchi* and *Leptomastidea abnormis*, respectively, per week from July 30 to October 8, 1999 (Table 1a).

OBJECTIVE 3.

Pre-treatment Data (prior to parasite releases or ant control)

Objectives have been met with respect to obtaining pre-treatment data (prior to parasite release) which provides a benchmark to compare with data obtained after the parasite releases. There are great differences in numbers of "native" or pre-existing parasites among all fields. There were also great differences in numbers of parasites recovered from the 2 species, with greater numbers of *Anagyrus* recovered than numbers of *Leptomastidea* (both findings on Table 2).

Data on VMB numbers were either zeros (two fields) or in very low numbers (two fields). Data on honeydew blotches on trunks and vines were also in relatively low incidence, although more readily detectable than live VMB. In general, the levels of honeydew blotches corresponded with the levels of live VMB. That is, honeydew blotches were notably higher in locations where VMB were found. Very low incidence (or none) of honeydew blotches were found in fields where there were zero live VMB.

Pitfall traps and visual time searches provided the best methods for assessing ant activity on the ground and in the vines. In visual observations, numbers of ants were also most closely correlated with sites where live VMB were found. Lowest (nearly zero) incidences of ants were from sites where there was zero live VMB and low incidence of honeydew blotches. In the absence of live VMB, ants were more abundant in areas where there was greater incidence of honeydew blotches.

Samples after parasite releases

The numbers of the VMB parasite *Leptomastidea* increased significantly in all 4 vineyards, most notably in parasite release areas (Table 4). *Leptomastidea* were recovered in 10 times greater numbers after releases compared with numbers prior to releases. *Leptomastidea* also increased in numbers as the season progressed. High levels of *Leptomastidea* continued up to more than two months after the last parasite releases on 8 October (Table 4). These results show that *Leptomastidea* are surviving and reproducing in the vineyards despite high day temperatures during August-October, and cold nights in November. The increase in numbers in non-release areas also indicates significant movement of these parasites from release to non-release areas. (Table 4).

We believe that the relatively low numbers of the VMB parasite *Anagyrus* recovered from release versus non-release areas in 1999 reflects the unusually high temperatures in the Coachella Valley from August through October. High temperatures are known to be detrimental to *Anagyrus*. However, in results from previous years, numbers of *Anagyrus* were equal to or better than those of *Leptomastidea*. Those results were taken from February through May when temperatures are relatively mild in the Coachella Valley.

Data collected in 1999 in our samples for live VMB, honeydew blotches, and ants were similar to those reported above under pre-treatment results. In samples taken approximately 2 and 4 weeks after the Lorsban ant treatment we found significant reductions in numbers of ants from treated vs untreated plots (Table 3). There were no significant reductions in numbers of parasites (*Anagyrus* or *Leptomastidea*) from treated vs untreated plots (Table 5).

A close relationship between VMB and ant activity has been documented, supporting the expectation that effective ant control will enhance parasite impact against VMB.

January to March 2000:

Objective 1: A field day was held on 17 March at Tudor ranch (one of our cooperators) to describe the VMB biological control program in the Coachella Valley. We presented objectives results and future plans. Representative of all personnel involved in this project were present and participated in the presentation (FAR Insectary, UCR research and extension, and all grower collaborators plus C. Hunter DPR, and J. Schrader from Riverside Co. Agric Com., PCA's from several companies, and several growers.

Objective 2: Modifications and improvements were continued in testing plant materials for rearing VMB and refinement of equipment to further reduce contamination of cultures.

Production and releases of *Anagyrus* is proceeding very well as reflected in weekly releases made beginning the first week of February (Table 1b). Except for the week of 4 February, over 192,000 *Anagyrus* have been released weekly over 7 weeks. The over-all average is more than 288,000 *Anagyrus* per week over the last 7 weeks.

Full scale releases of *Leptomastidea* have been variable. Regular full scale production of this parasite has been delayed because of recurring problems with equipment regulating relative

humidity. In 4 separate weeks when there were no humidity problems, more than 96,000 *Leptomastidea* were released each of those 4 weeks (Table 1b).

By the end of March we expect to be releasing weekly a minimum of 100,000 *Anagyrus* and 100,000 *Leptomastidea* in the Coachella Valley.

Objective 3: Two applications of Lorsban (one in February and one March) were made for ant control in designated plots on all 4 vineyards.

Low or 0 numbers of ants are being found in visual counts of ants in ant-treated plots. Significantly greater numbers of ants are being counted in non-treated plots.

VMB are beginning to appear and increase in numbers although levels are still relatively low. From counts in several fields there is a trend indicating notably higher levels of VMB in fields where commercial treatments were applied last year, and where we have not released parasites nor applied materials for ant control. Lowest numbers of VMB tend to be in plots treated with Lorsban for ants and/or where parasites were released.

Numbers of male VMB collected from yellow cards are closely correlated with the numbers of VMB in the 4 fields samples.

Numbers of parasites have been recorded on yellow sticky cards from all plots beginning the last week in January. Data are presently available only for pre-release collections (Table 6). Other cards are being counted and processed.

Data from pre-release samples show moderate levels of *Anagyrus* indicating reasonable survival over winter, and movement of the surviving parasites throughout fields and plots prior to releases this year. The data are strongly biased in favor of males.

Leptomastidea were recovered from pre-release samples in low numbers suggesting poor survival of winter conditions and/or emergence perhaps at a later date when temperatures are higher.

DISCUSSION:

Because of the funding date, work on Objectives 2 and 3 began after harvest and toward the end of the growing season during the first year of funding. Nevertheless, the system for production of parasites has been greatly improved and the results are that up to more than 25 times as many parasites are being produced as we had anticipated from previously using the traditional rearing methods. The lower cost of producing greater numbers of parasites with reduced contamination provides a stronger potential for biological control colonization and/or augmentation programs (Luck et al, 1992).

Results are incomplete for several reasons. After harvest (July), VMB retreat to refuge areas under the bark or to the roots. They become extremely difficult to sample under these conditions. Because of the low numbers of VMB after harvest the principal impact from our

studies will be demonstrated next spring when VMB, parasites, and ant activity is optimal. At the present stage, these preliminary results provide a strong basis for expecting significant impact from parasite releases against VMB in the spring when parasites are most effective. In addition to producing greater numbers of 2 types of parasites, we will also have effective reduction in ant activity without affecting parasite numbers to enhance the parasites' effectiveness. At the end of fiscal Year 1 (May 2000) we will have preliminary data on the impact from mass release of parasites on mealybug populations during the season, on over-wintering populations, and on yields. A minimum of two full seasons will be needed to estimate results from seasonal and between year's variation, for Objectives 2 and 3.

For the purpose of this report, it should be noted that funds from several sources were awarded for the rearing and releasing of the parasites, for the ant studies, and for evaluations. Additional funding to that provided by DPR was provided by the California Desert Grape Administrative Committee (CDGAC), the California Grape Commission and the Cooperative State Research, Education, and Extension Service of the USDA.

SUMMARY AND CONCLUSION:

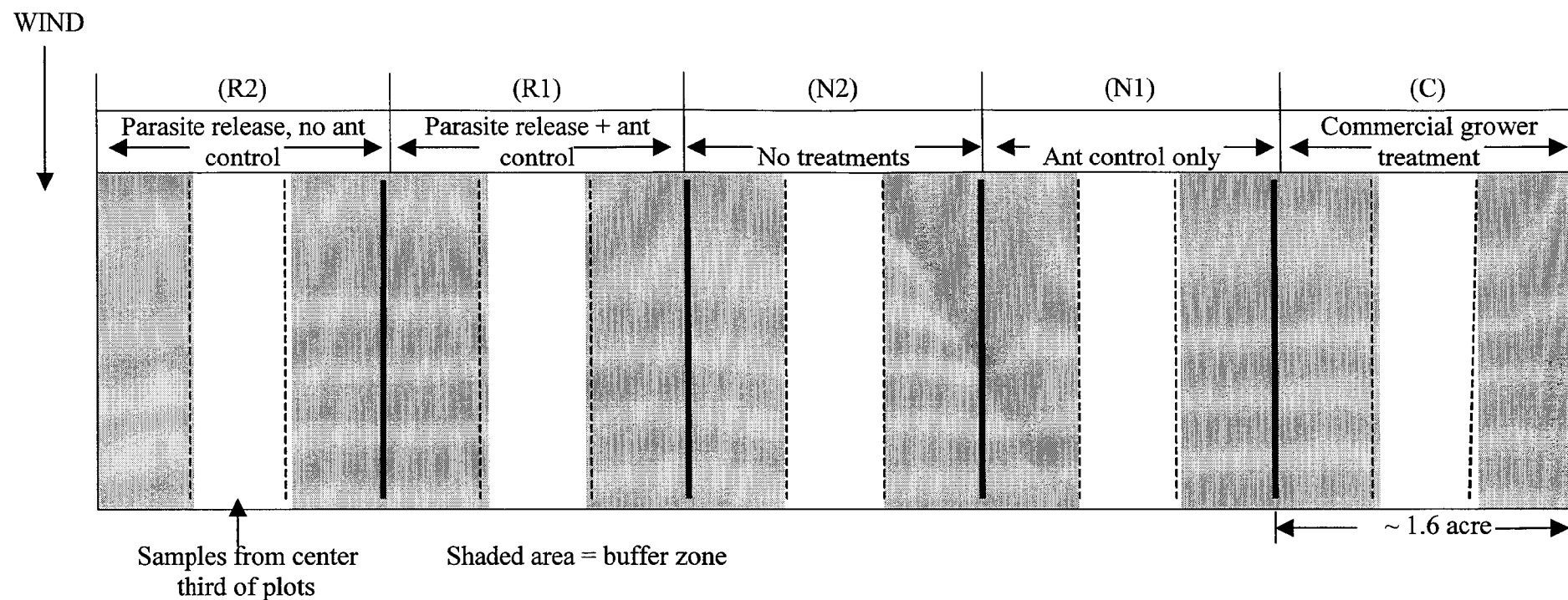
Significant progress has been made in each of the 3 objectives in this proposal. In objective 1, a group of collaborators has provided all resources needed to establish viable study/demonstration sites. In objective 2, insectary parasite production techniques have been modified resulting in a great increase in numbers of parasites produced and released, also with significant reduction in the potential for contamination of insectary rearing colonies. The significant increase in insectary production of parasites greatly enhances the potential to reduce VMB damage with biological control colonization and/or augmentation programs. In objective 3, we have demonstrated significant increases in *Leptomastidea* recovered following parasite releases under extremely hot temperatures. We have documented a close relationship between VMB and ant activity, supporting our expectation that effective ant control will enhance parasite impact against VMB. We have tested several baits for ant control and are collecting data needed to obtain registration for use in agricultural areas. We have designed a special spray rig that allows application of registered insecticides in such a manner to minimize impact on parasites.

The strong support from collaborator growers, the significant increase in insectary parasite production and field releases, the notable increase in *Leptomastidea* following parasite releases under extremely hot temperatures, and the development of an effective ant control method, in total provide a strong basis for high expectations of effective control of VMB in the spring preceding harvest next year. That is also the period when the VMB parasites are at maximum effectiveness.

LITERATURE CITED

- Berlinger, M. J. (1973a).** Effect of relative humidity and temperature on the survival of *Clausenia josefi* (Hymenoptera: Encyrtidae). *Entomologia Experimental et Applicata* 16: 373-79.
- Berlinger, M. J. (1973b).** Biological studies of *Clausenia josefi* (hymenoptera: Encyrtidae), a parasite of *Planococcus vitis*. *Entomophaga*. 18 (3): 297-86.
- Berlinger, M.J. (1977).** The Mediterranean vine mealybug and its natural enemies in southern Israel. *Pyhytoparasitca*
- Cox, J.M. (1986).** Planococcine mealybugs of economic importance from the Mediterranean Basin and their distinction from a new African genus (Hemiptera: Pseudococcidae). *Bull. Ent. Res.* 76: 481-489.
- Gill, R.J. (1994).** The vine mealybug (*Planococcus ficus*): a mealybug new to California. USDA Report/Letter to Eldon Reeves (Riv.Co. Aa. Commissioner's Office).
- González, D. 1998.** Naturally-occurring biological control, the most important component in crop protection programs. P. 183-186. *In*: Calif. Conf. Biol. Control Innovation in Biol. Control Research (M. Hoddle ed.) Berkeley, Calif.
- Klotz, J. H., Greenberg, L. and E. C. Venn. 1998.** Liquid boric acid bait for control of the Argentine ant (Hymenoptera: Formicidae). *J. Econ. Entomol.* 91:910-914.
- Luck, R., J. Morse, P. Haney, H. Gjriftiths, J. Barcinas, T. Roberts, E. Grafton-Cardwell, and N. O'Connell. 1992.** Citrus IPM – It Works. *Calif. Grower* 16 (4): 25-27.
- Myartseva, S.N. (1984).** Natural enemies of the family Encyrtidae (Hymenoptera, Chalcidoidea) in turkmenistan and scientific bases for their use in plant protection. *Izvestiya akademii Nauk Turkmenskoi SSR, Biologicheskikh Nauk* (No. 2): 23-30.
- Myartseva, S. N. and O. D. Nyazov. (1986).** Vine mealybug and its natural enemies in Turkmenistan. (V. A. Triapitzyn, Ed.) Ashkhabad: Ylym Publ. 92 pp.
- Nixon, G.E.J. (1951).** The association of the ants with aphids and coccids. *Commun. Inst. Ent.*, London.
- Phillips, P. and C. Sherk. 1991.** To control mealybugs, stop honeydew seeking ants. *Calif. Agric.* 45: 26-28.
- Reierson, D. A., Rust, M. K., and J. Hampton-Beesley. 1998.** Monitoring with sugar water to determine the efficacy of treatments to control Argentine ants, *Linepithema humile* (Mayr). *In* Proceedings of the National Conference on Urban Entomology, San Diego, Calif.
- Rosen, D. and Y. Rossler. (1966).** Studies of an Israel strain of *Anagyrus pseudococci* (Girault) Hymenoptera: Encyrtidae). I. Morphology of the adults and development stages. *Entomophaga* 11: 269-77.
- Shorey, H.H., L.K. Gaston, R.G. Gerber, P.P. Phillips, and D.L. Wood. (1992).** Disruption of foraging by Argentine ants, *Iridomyrmex humilis* (Mayr) (Hymenoptera: Formicidae), in citrus trees through the use of semichemicals and related chemicals. *J. Chem. Ecol.* 18(11): 2131-142.
- Triapitzyn, V. A. (1989).** Parasitic Hymenoptera of the Family Encyrtidae of the Palaearctics Leningrad, Nauka, Leningrad Division. 488pp.
- Wheeler, G. C. and Wheeler, J. N. (1986).** The ants of Nevada. A publication of Natural History Museum of Los Angeles Country, Allen Press, Lawrence, KS.

Fig. 1. Schematic Diagram of Experimental Design (field-plot arrangement)



Plots arranged randomly in each field

No insecticide applications up-wind

- 1 = ant control
- 2 = no ant control
- R = parasite release
- N = no parasite release
- C = commercial applications

Fig. 2. Spray-rig for Lorsban to control *F. perpilosa* in grape vineyards

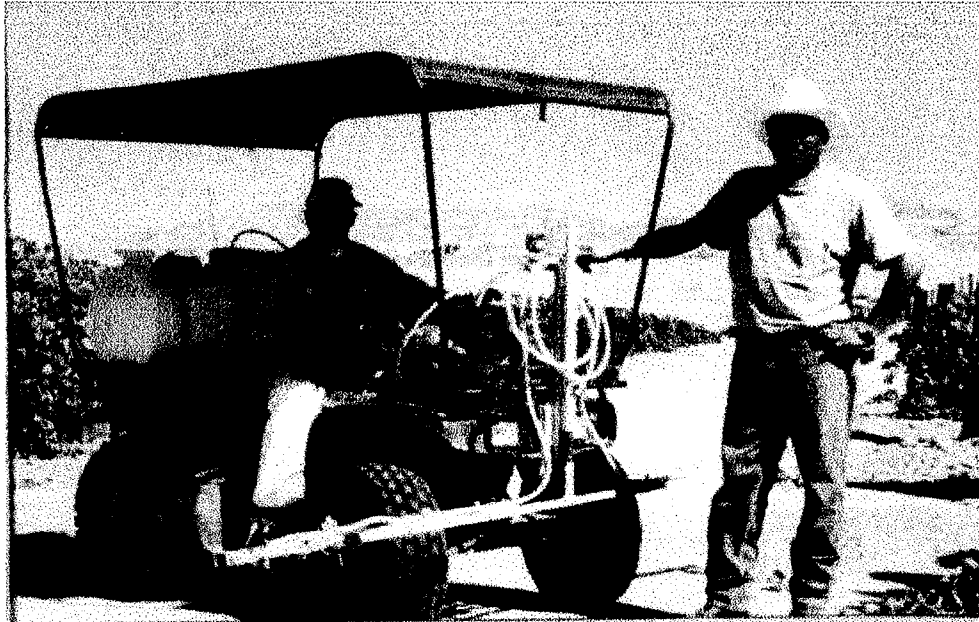


Fig. 3.

TIMELINE - YEAR 1 (1999)

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Objective 1 Formation of IPM Group				Completed								
Progress Review				First Meeting						Second Meeting		
Evaluation of IPM Group										Completed		
Objective 2				Begin rearing		Begin releases	Releases	Releases	Releases	Releases	End rearing	
Objective Ant Control sprays							Collect Data	Collect Data	Collect Data	Collect Data	Collect data	Collect data
Select Plots										Prepare Progress Report		

TIMELINE - YEAR 2 AND 3 (2000 - 2001)

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Objective 1 Formation of IPM Group		Evaluation Complete	Final DPR Report									
Progress Review			Field Day			First Meeting				Second Meeting		
Objective Begin rearing		Begin releases	Releases	Releases	Releases	Releases	Releases	Releases	Releases	Releases	End rearing	
Objective 3		Collect Data	Collect Data	Collect Data	Collect Data	Collect Data	Collect Data	Collect Data	Collect Data	Collect Data	Collect Data	
					Harvest Plots	Harvest Plots			Progress report			Disseminate Results and Encourage Implementation

Table 1. Parasite releases¹ and total production² by F.A.R. Insectary

a. 1999

<u>DATE</u>	<u>ANAGYRUS</u>		<u>LEPTOMASTIDEA</u>	
7/30/99	24,000 ¹	(68,500) ²	12,000 ¹	(28,700) ²
8/4/99	12,000	(90,500)	10,000	(34,000)
8/20/99	60,000	(68,800)	72,000	(86,000)
8/26/99	48,000	(121,000)	72,000	(125,000)
9/2/99	48,000	(88,000)	96,000	(106,000)
9/9/99	48,000	(78,000)	96,000	(99,000)
9/17/99	48,000	(79,000)	96,000	(110,000)
9/23/99	72,000	(77,000)	96,000	(101,000)
9/30/99	48,000	(56,000)	72,000	(94,000)
10/8/99	24,000	(46,000)	48,000	(96,000)
TOTAL	432,000¹	(772,800)²	670,000¹	(879,700)²

b. 2000

2/4/00	64,000	(224,000)	0	(98,000)
2/11/00	192,000	(435,000)	0	(79,000)
2/18/00	192,000	(452,000)	96,000	(120,000)
2/25/00	288,000	(408,000)	96,000	(129,000)
3/3/00	192,000	(354,000)	96,000*	(75,000)
3/10/00	384,000	(590,000)	0	(81,000)
3/17/00	384,000	(660,000)	0	(136,000)
3/24/00	384,000	(660,000)	96,000	(136,000)

* Numbers released included production also from previous week.

Table 2. Parasites* recovered from yellow sticky traps in 5 plots each in 4 fields prior to parasite releases in 1999

Future Treatments** (all untreated as of this date)																						
Vinyard	N1				N2				R1				R2				C				Totals	
	A		L		A		L		A		L		A		L		A		L			
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂		
Walker Tot.	7	1	0	0	3	2	0	0	7	0	0	0	2	3	0	0	8	1	0	0	A 34	L 0
	8		0		5		0		7		0		5		0		9		0			
Bianco Tot.	26	14	1	1	25	3	1	10	14	3	0	0	30	13	1	6	14	4	3	9	146	32
	40		2		28		11		17		0		43		7		18		12			
Fenn Tot.	41	22	0	0	35	66	1	1	60	49	0	0	54	71	1	0	35	7	0	0	440	3
	63		0		101		2		109		0		125		1		42		0			
Tudor Tot.	35	30	0	0	38	130	0	0	68	124	0	0	28	41	0	0	50	39	0	0	583	0
	65		0		168		0		192		0		69		0		89		0			
Totals	176		2		302		13		325		0		242		8		158		12		1203	35

* A= Anagyrus; L = Leptomastidea

** 1 = ant control; 2 = no ant control N = no parasite releases; R = parasite releases C = commercial treatment

Table 3. Mean number of ants per pitfall trap and visual count. 1999.

Pitfall Trap Data						
<u>Ant Control</u> ¹	<u>7/30</u>	<u>8/13</u>	<u>9/10</u>	<u>9/24</u>	<u>10/8</u>	
No	41.4	48.3	72.4	87.7	51.1	
Yes	86.6	97.4	79.1	8.4	17.7	
Significance of F ²	ns	ns	ns	***	*	
Time Search Data						
<u>Ant Control</u>	<u>7/29</u>	<u>8/12</u>	<u>9/10</u>	<u>9/24</u>	<u>10/7</u>	
No	15.8	11.6	14.4	15.9	13.9	
Yes	12.9	14.1	25.5	2.7	4.5	
Significance of F ²	ns	ns	ns	***	**	

¹ Ant control treatment applied 13 September 1999.

² ANOVA statistical analysis of log 10 (ants per trap + 1). Ns, *, **, *** = not significant, $P \leq 0.05$, 0.01, 0.001, respectively.

Table 4. Total Number of Parasites Recovered from yellow sticky traps 1999.

Pre-release (end July)	1 st		2 nd		3 rd		4 th		5 th		6 th		7 th		8 th		
	Recovery (beg Sept)		Recovery (mid Sept)		Recovery (beg Oct)		Recovery (mid Oct)		Recovery (beg Nov)		Recovery (mid Nov)		Recovery (beg Dec)		Recovery (mid Dec)		
	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	
NR* ("native")																	
Anagyrus																	
Walker	34	6	6	6	8	11	11	13	18	56	47	100	108	179	191	123	87
Bianco	146	69	45	58	45	44	60	56	60	118	133	87	72	74	112	45	77
Fenn	440	57	32	31	24	86	57	59	45	63	30	38	19	20	18	17	5
Tudor	583	43	37	26	25	37	36	41	24	35	83	87	156	57	125	13	15
TOTALS	1203	175	120	121	102	178	164	169	147	272	293	312	355	430	446	198	184
Leptomastidea																	
Walker	0	102	8	70	14	145	12	163	33	223	66	173	44	347	78	175	54
Bianco	32	93	39	168	39	152	36	373	106	162	64	111	45	172	67	71	18
Fenn	3	63	4	83	9	244	10	149	8	99	15	40	18	34	9	20	0
Tudor	0	78	0	70	3	91	8	85	9	50	13	125	18	54	7	2	0
TOTALS	35	336	51	391	65	629	66	770	156	534	158	449	125	607	161	268	72

*NR only figures from non-release plots (do not include counts from commercial plot)

Table 5. Number of *Anagyrus* and *Leptomastidea* collected from yellow sticky traps in plots treated (with Lorsban) and not treated for ant control 1999.

Parasite species	Parasite Numbers			
	Pre-treatment samples*	Days after Lorsban application		
		2-10	16-24	30-37
Anagyrus				
Treated	276	249	209	358
Not treated	242	93	107	207
Leptomastidea				
Treated	391	351	406	335
Not treated	452	334	520	357

* Totals from 2 sample dates.

Table 6. Parasites* recovered from yellow sticky traps in 5 plots each in 4 fields. Pre-release, 2000

Vinyard	Treatments**											
	N1		N2		R1		R2		C		Totals	
	A ♀ ♂	L ♀ ♂	A ♀ ♂	L ♀ ♂	A ♀ ♂	L ♀ ♂	A ♀ ♂	L ♀ ♂	A ♀ ♂	L ♀ ♂		
Walker Tot.	3 22 25	0 1 1	4 17 21	2 7 9	2 44 46	1 4 5	2 27 29	2 3 5	1 21 22	0 4 4	A 143	L 24
Bianco Tot.	7 45 52	0 1 1	9 58 67	1 6 7	11 55 66	2 1 3	13 75 88	7 13 20	5 18 23	2 8 10	296	41
Fenn Tot.	7 18 25	0 0 0	4 7 11	0 0 0	9 23 32	0 0 0	6 7 13	2 0 2	15 25 40	0 0 0	121	2
Tudor Tot.	2 27 29	0 0 0	7 35 42	0 0 0	5 42 47	1 0 1	3 15 18	0 0 0	8 33 41	0 0 0	177	1
Totals	131	2	141	16	191	9	148	27	126	14	737	68

* A= Anagyrus; L = Leptomastidea

** 1 = ant control; 2 = no ant control N = no parasite releases; R = parasite releases
C = commercial treatment

	Yelo Traps		Dates for preceeding 2 parasite releases pre-release
	Placed in field	Collected	
Walker	1/21/00	2/4/00	
Bianco	1/21/00	2/4/00	
Fenn	1/21/00	2/4/00	
Tudor	1/21/00	2/4/00	